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Epidermal Laser Stimulation of Action Potentials in the Frog Sciatic Nerve

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14. ABSTRACT

Measurements of laser stimulated action potentials in the sciatic nerve of leopard frogs (*Rana pipiens*) were made using two infrared lasers. The dorsal sides of the frog's hind limbs were exposed to short-pulsed 1540 nm and 1064 nm wavelengths at three separate spot sizes: 2 mm, 3 mm, and 4 mm. Energy density thresholds were determined for eliciting an action potential at each experimental condition. Results from these exposures showed similar evoked potential thresholds for both wavelengths. The 2-mm diameter spot sizes yielded action potentials at irradiance levels almost double that seen with the larger beam sizes.

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ABSTRACT

Measurements of laser stimulated action potentials in the sciatic nerve of leopard frogs (*Rana pipiens*) were made using two infrared lasers. The dorsal sides of the frog's hind limbs were exposed to short-pulsed 1540 nm and 1064 nm wavelengths at three separate spot sizes: 2 mm, 3 mm, and 4 mm. Energy density thresholds were determined for eliciting an action potential at each experimental condition. Results from these exposures showed similar evoked potential thresholds for both wavelengths. The 2-mm diameter spot sizes yielded action potentials at irradiance levels almost double that seen with the larger beam sizes.

1. Background

Electrical stimulation is commonly used to stimulate action potentials in neurons for both medical and research applications. Electrical signals are applied to a nerve, initiating the voltage change that will start a chain reaction along the axon. Once began, the signal is passed along the axonal tract much the same way as a "natural" action potential. Unfortunately, electrical stimulation systems possess characteristics that create problems in this kind of work. Besides low spatial specificity (electrical stimulation will activate several nerve tracts simultaneously), difficulties include tissue damage from electrode installation and unnatural action potential responses (Izzo, Pathria, Suh, Whitlonb, Jansen, & Richter, 2006). Recent studies have found that a laser source can be used to induce an action potential in the nervous system as well as, if not better than, electrical methods. Kao et. al. have shown that there are few differences between optical and electrical stimulation on the activation of the nerves (Kao, Wells, & Jansen, 2005). Laser excitation of neural activity provides a contact-free, spatially selective, artifact-free method of stimulation without incurring tissue damage (Kao, Wells, & Jansen, 2005). The small spot sizes used by laser systems allow for pin point accuracy when stimulating nerve tracts and the low irradiance levels help to minimize introduction of extra energy into the action potential response. The amplitude of an action potential is directly proportional to the strength of its triggering event so these lower energies used by laser stimulations are very important in getting more "natural" results, and achieving greater specificity.

Low energy levels can be utilized by lasers because their energy deposition can be more directly applied to nerve receptors by using wavelengths that penetrate to the depth that the receptors are located at. Energy deposition from near-infrared radiation is concentrated in the dermal layer where the majority of neural receptors are located. (See Figure 1) As the wavelength increases, the depth of penetration lessens, until just the outer layers of the epidermis are being irradiated. As presented by Welch, the penetration depth of a Q-switched Nd:YAG laser at 1064-nm is about 1 cm while the penetration depth of a Q-switched Er:glass laser at 1540-nm is around 1 mm (Welch & van Gemert, 1995). This selective energy concentration minimizes trauma to surrounding tissues and allows for maximal stimulation of nerve endings.

In addition to direct stimulation of nerve receptors, indirect neural stimulation can occur as a result of a laser exposure at the skin surface. This irradiation will induce a temperature rise in the tissue, causing the skin's thermal and pain receptors (nociceptors) to fire.

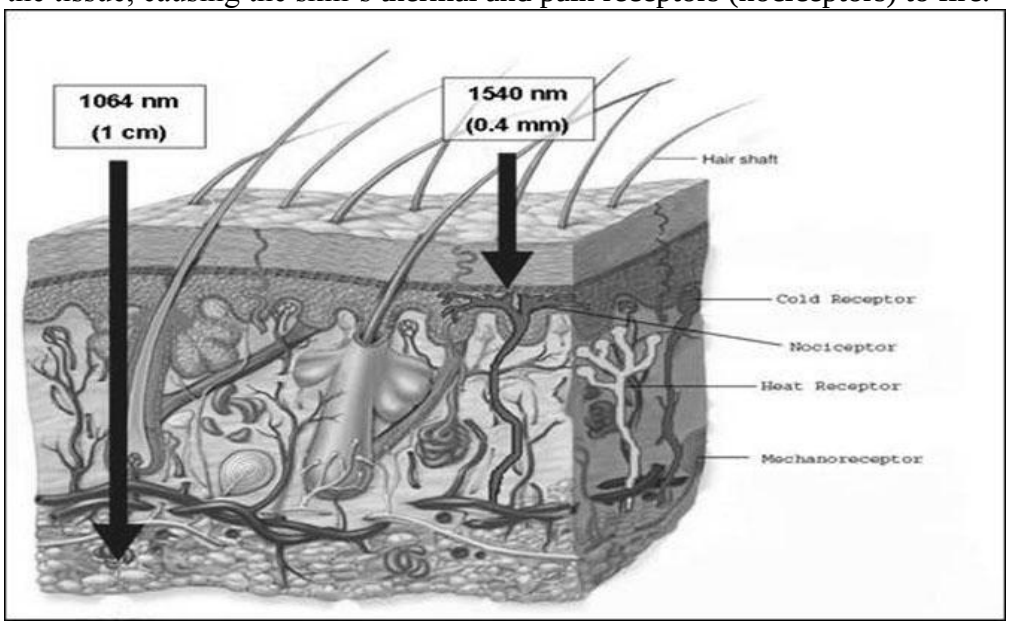

Figure 1: Illustration of laser energy penetration and nerve receptor locations in skin layers.

Thermal receptors are located in the dermis of the skin and nociceptors are located in both the dermis and the deep epidermal layer (see Figure 1). While most heat receptors activate at ranges just outside of the body's normal temperature, the threshold for onset of a painful sensation is approximately 45^{o}C -- the temperature at which heat produces tissue damage (Hendry & Hsiao, 2003). Signals from each of these receptors travel along peripheral nerves until they reach the central nervous system. Activation of distal receptors can be monitored by measuring impulses at various points along this neural system.

2. Objectives

The first goal of this work was to perform an experimental evaluation of the electro-potential response of neural receptors to pulsed laser exposures.[†] The second goal was to determine the relationship between stimulated potentials and laser wavelength relative to absorption properties of the tissue. These goals were tested during an experiment that was performed on Leopard Frogs. An electrode was placed in the sciatic nerve of the frogs to record action potentials elicited from laser exposures on the skin of the calf. Three spot

[†] All experiments were conducted at Air Force Research Laboratories Human Effectiveness Directorate Optical (RHDO) radiation branch following approval by the Institutional Animal Care and Use Committee (Protocol HEDO-06-12).

sizes and two different wavelengths were used in the study. The data was then analyzed to determine threshold values for action potential stimulations.

3. Methods and Materials

3.1 Leopard Frogs

In vivo sciatic nerve experiments were performed using Leopard Frogs (*Rana pipiens*) from the Carolina Biological Supply Company in North Carolina. The frogs ranged in torso length from 3 to 4 inches and were euthanized via a double pithing technique. To maintain a constant body temperature during the experiment, the cold-blooded frogs were placed on a saline bag that had been warmed to approximately 20-22 ˚C. This was to ensure that the nerve receptors remained within their effective ranges and that the frogs were not negatively affected by cold ambient temperatures in the lab.

Since the subject was an amphibian, the water content of the skin was very high. In order to minimize variability in the data obtained during this experiment, saline was periodically applied to the skin to maintain hydration. Excess solution was blotted off using gauze.

3.2 Nerve Preparation

Nerve preparation started by making a centrally located incision from the knee to the upper thigh, removing the skin from the dorsal side and exposing the trunk of the nerve at the knee. Muscular fascia was incised and removed to expose the rest of the nerve. A piece of latex was placed between the nerve and the underlying muscle in order to minimize any collateral electrical signals. After the nerve was prepared, insulated stainless steel needle electrodes (Chalgreen Enterprises, Inc. 111-637-24TP, Disposable, Monopolar EMG Needle Electrodes, 37mm x 26 gauge) were inserted into the nerve located approximately 15 mm above the knee. Baseline data was collected to verify the system's isolation from other electrical sources and to ensure correct electrode placement. To initialize each experiment, the sciatic nerve was directly stimulated by the laser to verify that the electrode was reading correctly and that the nerve had not been damaged by the insertion. Compound nerve action potential (CNAP) responses were recorded with BioPac Systems Inc. MP100 interfaced to a computer running Acknowledge software v3.73. The CNAP is the algebraic sum of many individual "all-or-none" action potentials arising more or less simultaneously in a large number of individual axons (Physiology Dept., McGill University, 2005). All action potentials are measured using a differential medical amplifier and extracellular recording electrodes, which measure the summed electrical response of all excited axons in the nerve. The recordings for this project were manually triggered prior to the exposure and recorded 5 seconds of data. All signals were amplified 1000 times and electrically filtered with a 50 to 5000 Hz bandpass filter.

3.3 Laser Set-Up

Optical stimulation was performed using two infrared laser sources. A Q-switched Nd:YAG laser emitting 1064 nm was first used to verify experimental methods. The Nd:YAG laser was pulsed at a repetition rate of 10 Hz with a pulse duration of 15 ns. The energy of the laser was controlled using a half wave plate and a polarizing beam splitter, and the generated pulses were sampled using a 90/10 non-polarizing beamsplitter by an Ophir Laser Star energy meter using #1Z0230 power head. The laser was then directed into a faraday cage where it would be focused into the desired spot size using a 500 mm bi-convex lens. (Figure 2)

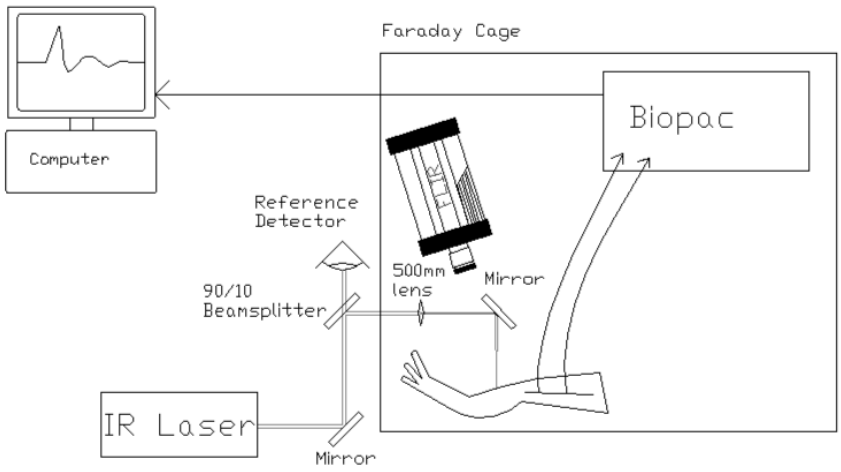

Figure 2: Generic schematic of experimental set-up.

Each infrared laser used possessed a Gaussian spatial beam profile. Beam diameters and profiles were measured using linagraph laser burn paper. Using only one pulse per exposure, three beam diameters were used during the experiment; 2 mm, 3 mm and 4 mm (*diameter measured at* e^{-2} *irradiance*).

Once the methods were verified with the Nd:YAG laser, an Er:Glass (Erbium-glass) laser emitting 1540-nm was employed to further evaluate optical stimulation and for statistical comparison at another wavelength (site forward). The Er:Glass laser was mechanically q-switched to a pulse duration of 55 ns. Due to the system's high energy, only 1 shot per minute is allowed. The energy of the laser is varied by adjusting the flash lamp energy. Again the beam was directed into the faraday cage and focused to the same spot sizes as with the 1064-nm Nd:YAG laser: 2 mm, 3 mm and 4 mm.

3.4 Probit Analysis

Probit analysis was developed in order to analyze discrete data collected by experiments involving threshold response rate in biological systems. This is computed using the EZ-Probit program designed by Dr. Clarence Cain and Capt. Lonnie Manning at Brooks City-Base in San Antonio, Texas (Cain & Manning, 1996). This method has been employed as

a statistical tool to determine the probability of dose-response curves for action potential (AP) responses in the sciatic nerve. In this case the thresholds probabilities are reported as AP_{50} or the dose which has a 50% probability of creating a response. The values presented here are for 100% probability, without consideration of additional experimental uncertainties. Also, the slope of the probit line is calculated between the ED_{84} and ED_{50} values. A high value for slope would represent high value for data certainty, with minimal sample-to-sample variability affecting results.

4. Results

Data showed considerable variability between the animals in respect to the pigmentation placement. While melanin has only a small role in energy absorption at infrared wavelengths, early skin exposures showed noticeable differences between skin damage threshold energy levels for dark and light skin patches. Thus, for greater consistency, only lightly pigmented skin data was used in the analysis. Each AP_{50} is represented in units of fluence (J/cm^2).

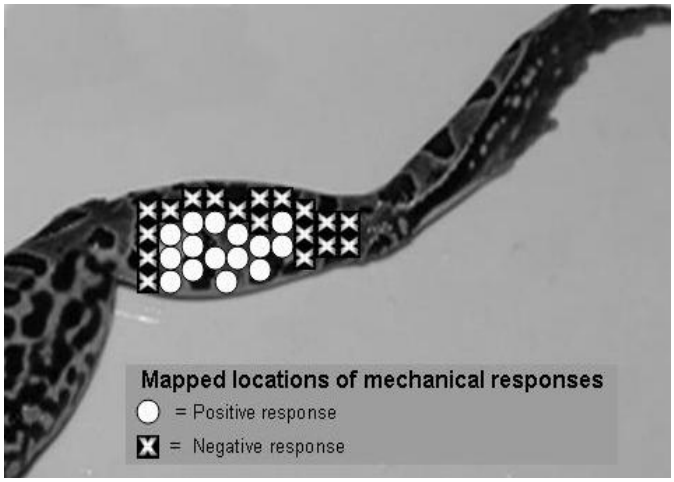

Figure 3: Mapped locations of frog response areas

A drawback of stimulating nerve receptors through the skin instead of directly on the nerve is that receptors are not uniformly located across an area. For example, in humans it is known that some areas such as the palms of the hands have very few nerve receptors, whereas other areas, like the tongue or face, have a large concentration of receptors. In order to get an idea of the distribution on the frog's leg, several frogs were subjected to a consistent irradiance level for multiple exposures across the area. The spots were randomly irradiated with one minute between shots so that the nerve had time to recover from each exposure. The energy used was high enough to elicit a visual muscular reflex so that it was not necessary to use the nerve probes. While each frog varied in the actual responses, Figure 3 shows the general nerve distribution found from this study. The circles were areas where consistent reflex responses were located while the X's show locations that did not give any reflexive actions.

Positive responses, such as that seen in Figure 4, were recorded for every parameter tested in this project. These viable action potentials obtained from the laser exposures are presented in Table 1. Measurements were taken from multiple subjects and calculated by combining all data for each spot size and wavelength.

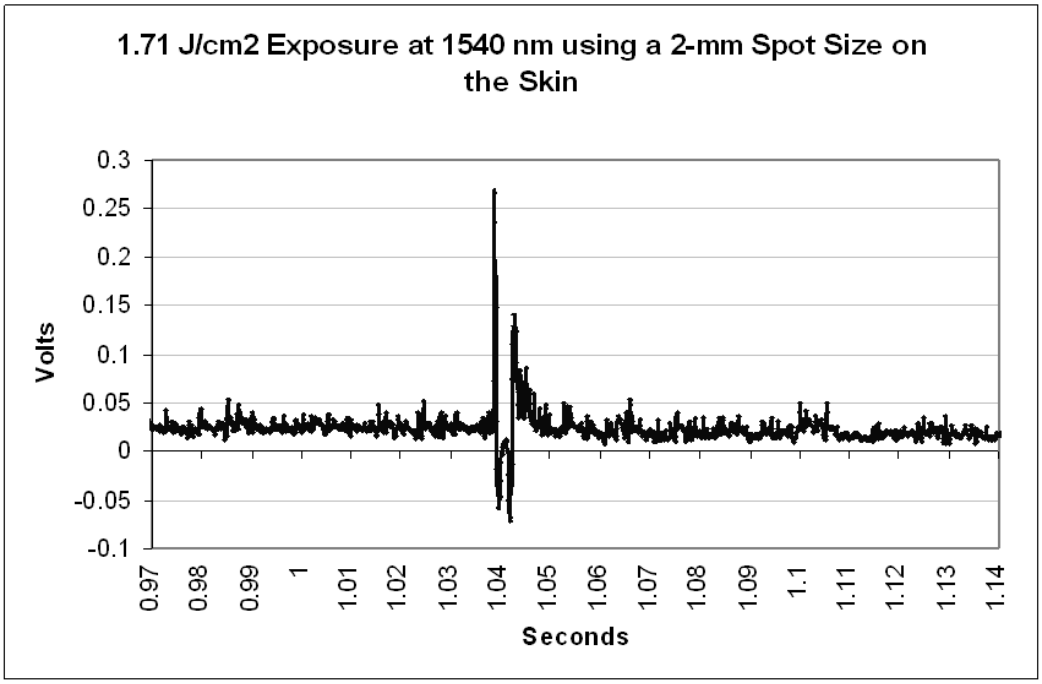

Figure 4: Action potential elicited from laser exposure on the frog's calf

ACTION POTENTIAL THRESHOLDS								
Spot Size	**1064 nm**				**1540 nm**			
	AP50	**UFL**	**LFL**	**SLOPE**	**AP50**	**UFL**	**LFL**	**SLOPE**
2 mm	**0.900**	**1.262**	**1.470**	**5.483**	**1.331**	**1.838**	**1.981**	**74.783**
3 mm	**0.497**	**0.557**	**0.443**	**25.910**	**0.449**	**0.712**	**0.195**	**3.423**
4 mm	**0.430**	**0.520**	**0.297**	**3.678**	**0.323**	**0.350**	**0.296**	**37.950**

Table 1: Action potential thresholds. UFL = upper fiducial limit. LFL = lower fiducial limit

The action potentials elicited demonstrated very similar activation trends among the 1064- and 1540-nm lasers. The 2-mm spot sizes required almost double the energy to reach threshold than the larger beams, regardless of which wavelength was used. (Figure 5 and Figure 6) This data also shows that the skin became damaged at levels below AP thresholds for the both wavelengths when using a 4-mm spot size. The most notable difference between the two lasers was that skin damage occurred below the action potential threshold when using the 3 mm beam at 1064 nm, but not at 1540 nm. (Figure 5 and Figure 6)

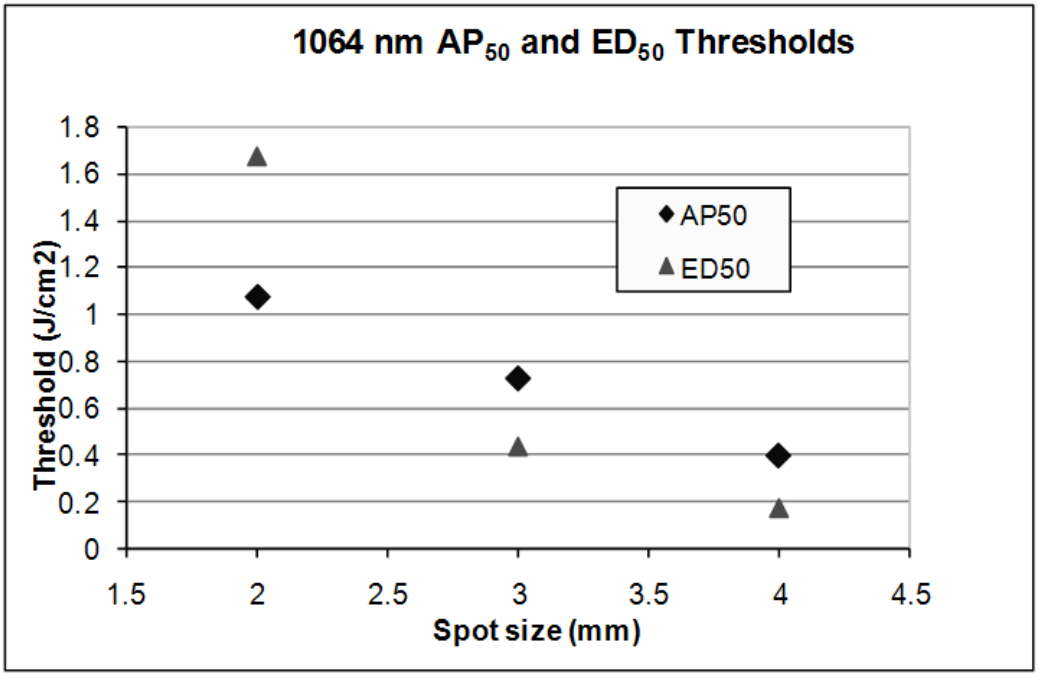

Figure 5: Skin damage and action potential thresholds at 1064 nm.

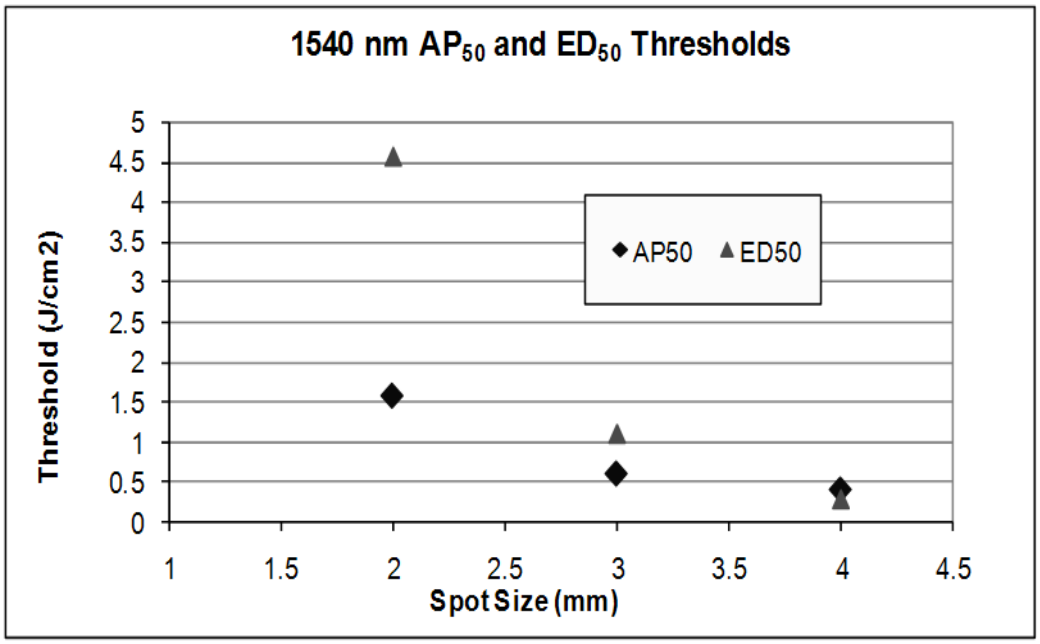

Figure 6: Skin damage and action potential thresholds at 1540 nm.

5. Discussion

A major impediment to this project was the skin's tendency to ablate, even at very low energy levels (0.169 J/cm^2). The ablation was inconsistent and varied depending on pigmentation and location of the exposure site.

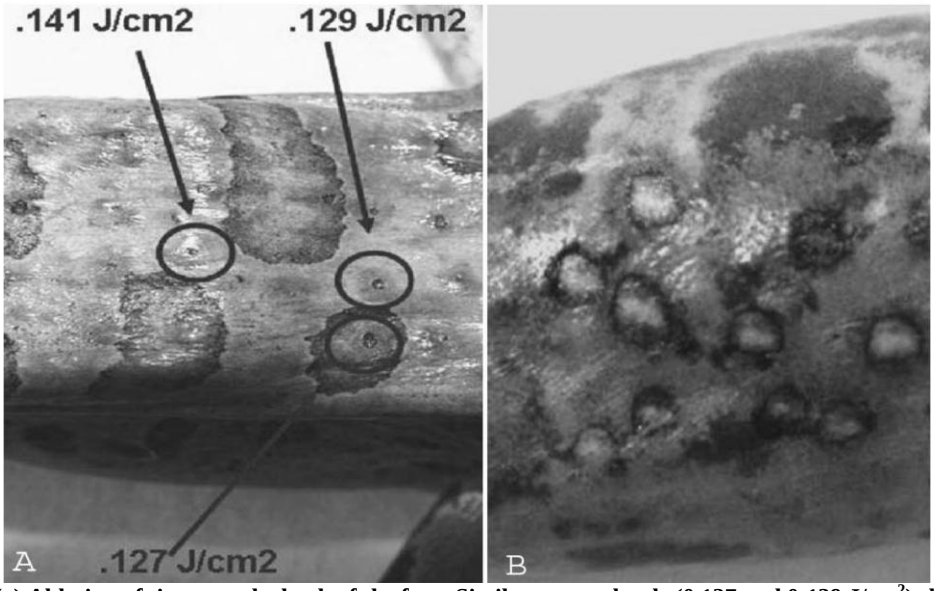

Figure 7: (a) Ablation of tissue on the back of the frog. Similar energy levels (0.127 and 0.129 J/cm^2) show larger amounts of damage for the more heavily pigmented areas than the lightly pigmented areas. (b) Ablation of skin on the dorsal surface of a frog leg. Note the black rings surrounding each crater, potentially indicating charring of the perimeter tissue from thermal effects. Exposure energies varied from 0.386 to 1.12 J/cm^2.

As seen in Figure 7a, the ablation was inconsistent and varied depending on pigmentation and location of the exposure site. Dark pigmented tissue required less energy and had larger ablation diameters. Thermal data from these areas show temperature rises as low as 0.689 ^{o}C so ablation due to thermal effects are most likely not the reason for this. Many of these low powered exposures did not exhibit the same charred responses around the crater perimeter as the ablations seen with high irradiance exposures. (Figure 7b) The most probably cause, although confirmation of this will require additional experimentation, would be photomechanical damage due to stress confinement. Thermoelastic expansion of tissue by pulsed laser will eject ablated material through stress wave recoil. Stress Waves are produced when optical energy is absorbed into an appropriate medium. If the irradiance is high enough, dielectric breakdown can occur which leads to the formation of high-pressure plasma and the production of large-amplitude stress waves in the tissue. (Dyer & Al-Dhahir, 1990) Shock wave damage effects are due to both compressive and tensile strain. The estimated stress confinement time for this experimental arrangement is 7 μs and 3 μs for 1064 nm and 1540 nm respectively, calculated using Equation 1. (Welch & van Gemert, 1995)

$$\tau_p < \frac{\delta}{\sigma}$$

Equation 1: The criterion for stress confinement is given by where τ_p is the laser pulse length, δ the penetration depth of laser light in tissue, and σ is the speed of sound in tissue.

As previously stated, the neural response was reliant on the location of the exposures site because of the distribution of nerve receptor endings. For exposures using a large spot size, areas with more thermal receptors showed responses at lower irradiance levels than exposures at sites that had just a few receptors. This could be due, in some part, to activation of more nerve receptors with the larger beam.

The action potential thresholds achieved were not very different, despite the distinct penetration depth.

While the parameters of this experiment did not provide any conclusive information, the data from the 4-mm 1540 nm exposures did provide some insight as to future possibilities for this kind of work. This larger spot size provides for a more linear temperature rise. Figure 8 shows the relationship between temperature rise and the probability of eliciting an action potential.

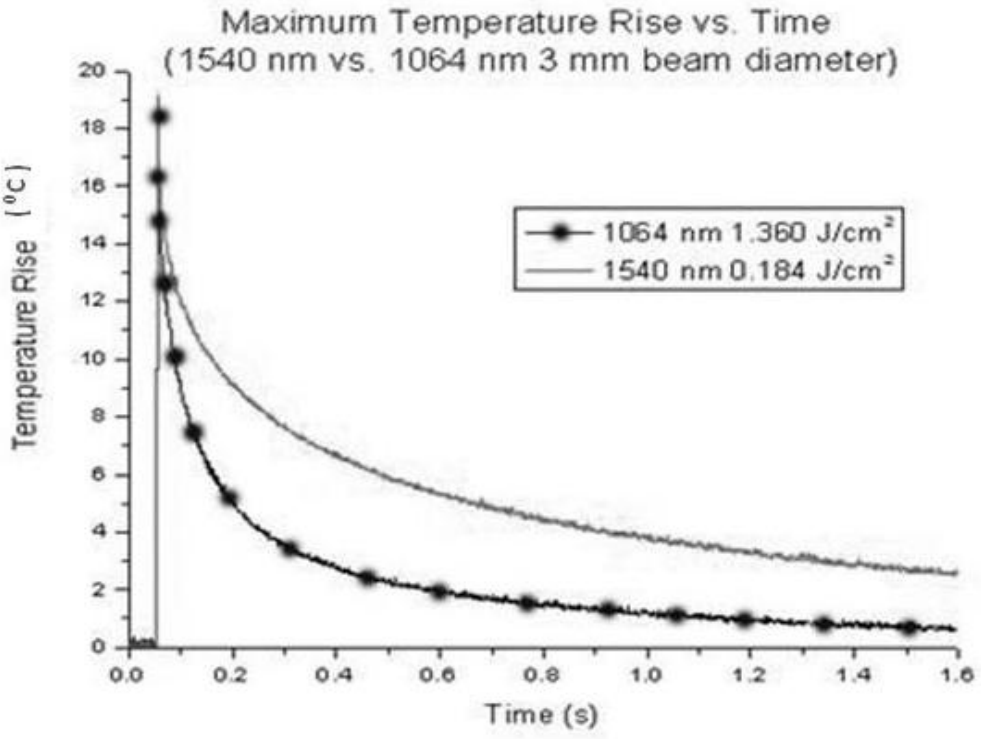

Figure 8: Cooling trend for exposures with 20° C temperature rise for 1064nm and 1540nm wavelengths.

It should be noted that the thickness of frog skin is much less than that of humans. The average combined thickness of the epidermis, dermis and subcutaneous layers measured in these experiments was 0.367 mm. This value is approximately the thickness of the human epidermis layer alone (Jiang & al., 2002). Therefore, it is difficult to draw direct correlation between results achieved in this study to any expected values for human response.

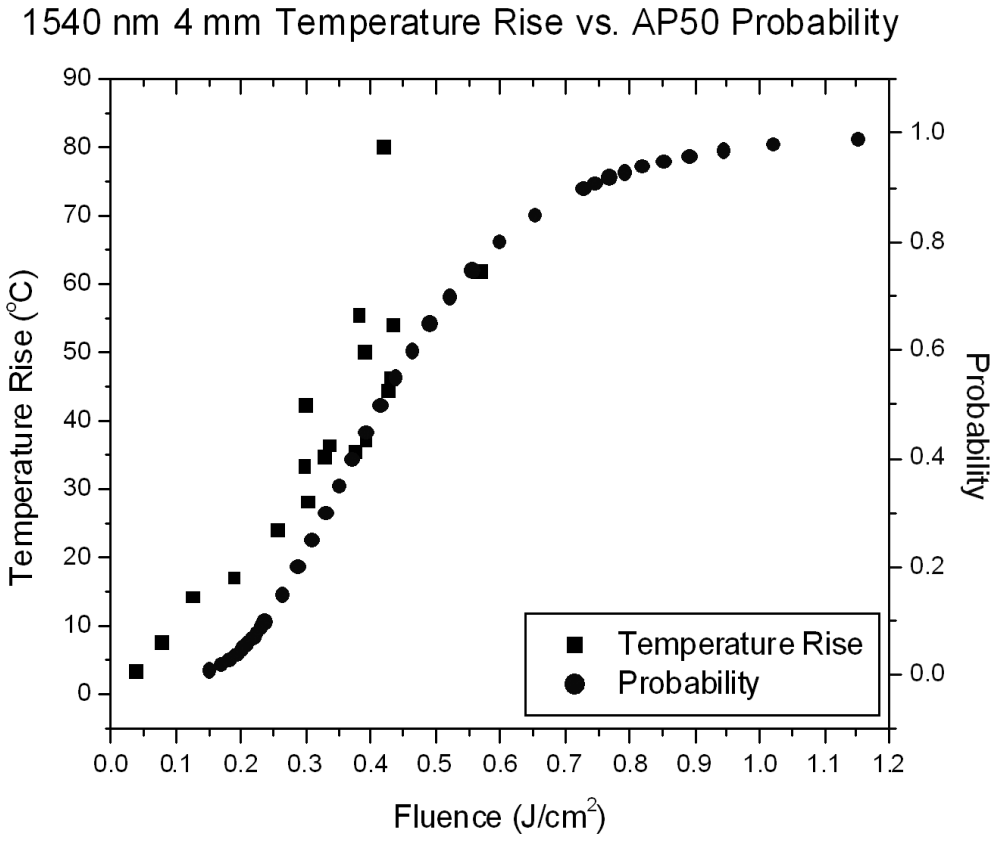

Figure 9: AP_{50} vs. temperature rise data for 1540 nm exposures with a 4 mm spot size.

6. Conclusions

The results presented in this document are only the beginning of a new line of research in the systematic characterization of neural stimulation with lasers. For this research we have studied the effects at two wavelengths and three individual beam diameters. The most significant finding provided by this study was that small beam diameters were needed to avoid tissue damage and cause stimulation. As the results show, larger beam diameters have much lower thresholds in terms of radiant exposure for both neural stimulation and skin damage. As laser beam diameters increase, the damage threshold decreases. The action potential threshold for the larger spots is lower, since the laser is stimulating a greater number of neurons. Therefore, based upon our findings, the ideal spot size would be 3 mm since it required lower laser energy to stimulate action potentials, and did so at irradiance levels below those that cause skin damage. It was shown that tissue ablation occurred well before the average surface temperature of the skin reached 100° C, which may be explained by laser induced breakdown or stress confinement mechanisms. Indeed, skin damage frequently occurred before action potentials were stimulated at beam diameters of 4 mm for each wavelength. This phenomenon will certainly require additional

studies to determine the exact mechanism of damage; whether it be thermal, mechanical, or a combination of the two.

It became obvious that the differences between frog skin pigmentation and morphology from that of humans makes them ill suited as human skin damage threshold models. A mammalian study may provide the necessary data to determine the best wavelength for creating action potentials without causing skin damage.

Finally, this study was conducted with two wavelengths common in the medical and photonics industries. Additional wavelengths should be studied to determine if different penetration depths or powers could yield more optimal results.

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